The effects of running exercise on oxidative capacity and PGC-1α mRNA levels in the soleus muscle of rats with metabolic syndrome.

AUTHOR(S):

Nagatomo, Fumiko; Fujino, Hidemi; Kondo, Hiroyo; Kouzaki, Motoki; Gu, Ning; Takeda, Isao; Tsuda, Kinsuke; Ishihara, Akihiko

CITATION:

Nagatomo, Fumiko ...[et al]. The effects of running exercise on oxidative capacity and PGC-1α mRNA levels in the soleus muscle of rats with metabolic syndrome.. The journal of physiological sciences : JPS 2012, 62(2): 105-114

ISSUE DATE:

2012-03

URL:

http://hdl.handle.net/2433/156783

Journal of Physiological Sciences (Original Research)

The effects of running exercise on oxidative capacity and PGC-1α mRNA levels in the soleus muscle of rats with metabolic syndrome

Fumiko Nagatomo[1] · Hidemi Fujino[2] · Hiroyo Kondo [3] · Motoki Kouzaki[4] · Ning Gu[5] · Isao Takeda[6] · Kinsuke Tsuda[7] · Akihiko Ishihara[1]

[1]Laboratory of Cell Biology and Life Science, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan

[2]Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, Kobe 654-0142, Japan

[3]Department of Food Sciences and Nutrition, Nagoya Women's University, Nagoya 467-8610, Japan

[4]Laboratory of Neurophysiology, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan

[5]Department of Life Science and Engineering, Harbin Institute of Technology, Harbin 15001, China

[6]Department of Physical Therapy, Takarazuka University of Medical and Health Care, Takarazuka 666-0162, Japan

[7]Laboratory of Metabolism, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan

Abstract

Skeletal muscles in animals with metabolic syndrome exhibit reduced oxidative capacity. We investigated the effects of running exercise on fiber characteristics, oxidative capacity, and mRNA levels in the soleus muscles of rats with metabolic syndrome (SHR/NDmcr-cp (*cp*/*cp*); CP). We divided 5-week-old CP rats into non-exercise (CP) and exercise (CP-Ex) groups. Wistar-Kyoto rats (WKY) were used as the control group. CP-Ex rats were permitted voluntary exercise on running wheels for 10 weeks. Triglyceride levels were higher and adiponectin levels lower in the CP and CP-Ex groups than in the WKY group. However, triglyceride levels were lower and adiponectin levels higher in the CP-Ex group than in the CP group. The soleus muscles in CP-Ex rats contained only high-oxidative type I fibers, whereas those in WKY and CP rats contained type I, IIA, and IIC fibers. Muscle succinate dehydrogenase (SDH) activity was higher in the CP-Ex group than in the CP group; there was no difference in SDH activity between the WKY and CP-Ex groups. Muscle proliferator-activated receptor γ coactivator-1α (PGC-1α) mRNA levels were higher in the CP-Ex group than in the CP group; there was no difference in PGC-1α mRNA levels between the WKY and CP-Ex groups. In CP-Ex rats, longer running distance was associated with increased muscle SDH activity and PGC-1α mRNA levels. We concluded that running exercise restored decreased muscle oxidative capacity and PGC-1α mRNA levels and improved hypertriglyceridemia in rats with metabolic syndrome.

Keywords: Adiponectin · Muscle fiber type · Obesity · Triglyceride · Succinate dehydrogenase activity

Abbreviations

CP	SHR/NDmcr-cp (*cp*/*cp*)
CPT-I	Carnitine palmitoyltransferase-I
GLUT4	Glucose transporter 4
HPRT	Hypoxanthine phosphoribosyltransferase
MCAD	Medium-chain acyl-CoA dehydrogenase
PGC-1α	PPARγ coactivator-1α
PPAR	Peroxisome proliferator-activated receptor
SCD-1	Stearoyl-CoA desaturase-1
SDH	Succinate dehydrogenase
SHR	Spontaneously hypertensive rat
TFAM	Mitochondrial transcriptional factor A
WKY	Wistar Kyoto

Introduction

Metabolic syndrome is closely related to physical inactivity and consumption of a high-fat and high-calorie diet. Increased energy intake is the major cause of excessive body weight and leads to increased blood pressure and glucose levels. Metabolic syndrome ultimately will develop into a lifestyle-related disease, e.g., cardiovascular disease and type 2 diabetes [1–3].

The skeletal muscles are the major site of insulin action and glucose metabolism; reduced muscle oxidative capacity impairs oxidative metabolism and increases the risk for the development of metabolic syndrome, lifestyle-related diseases, and associated complications [4]. The skeletal muscles in patients with type 2 diabetes contain a lower percentage of high-oxidative fibers than those in healthy individuals [5–8]. Furthermore, we observed that the soleus muscles in rats with type 2 diabetes contain a lower percentage of high-oxidative fibers than those in non-diabetic rats [9–12].

The skeletal muscle characteristics are regulated mainly by peroxisome proliferator-activated receptors (PPARs), e.g., PPARα, PPARδ/β, and PPARγ, and PPARγ coactivator-1α (PGC-1α) [13–15]. We had previously reported that the soleus muscles in rats with type 2 diabetes contain lower levels of PGC-1α mRNA than those in non-diabetic rats [16, 17]. Therefore, we concluded that low PGC-1α mRNA levels in the soleus muscles in rats with type 2 diabetes are associated with a low percentage of high-oxidative fibers.

In the present study, we hypothesized that running exercise would increase oxidative capacity and PGC-1α mRNA levels in the skeletal muscles of patients and animals with metabolic syndrome and lifestyle-related diseases. We examined the slow soleus muscles in rats with metabolic syndrome that were permitted voluntary exercise on a running wheel; specifically, we focused on fiber characteristics (including type distribution, cross-sectional area, and oxidative enzyme activity) and mRNA levels related to glucose and lipid

metabolism. We expected marked improvement in the oxidative capacity of the soleus muscles in rats with metabolic syndrome following running exercise. The soleus muscles in normal rats exhibit increased oxidative capacity and function at relatively low intensity for a long duration. The soleus muscles are required to function against gravity, e.g., to maintain posture and to walk [18]; this indicates that these muscles function most effectively during oxidative metabolism. We used the SHR/NDmcr-cp (*cp*/*cp*) (CP) rat as an animal model for metabolic syndrome. CP rats have a nonsense mutation in the leptin receptor and develop obesity, high blood pressure and glucose levels, hyperinsulinemia, and dyslipidemia during adult stages [19, 20].

Materials and methods

All experimental procedures and animal care were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health and the Japanese Physiological Society. This study was approved by the Institutional Animal Care and Experiment Committee of Kyoto University (Kyoto, Japan).

Experimental animals and treatment

Five-week-old male CP rats were divided into 2 groups: non-exercise (CP; $n = 6$) and exercise (CP-Ex; $n = 6$) groups. Age-matched male Wistar-Kyoto rats were used as the control group (WKY; $n = 6$). All rats were obtained from Japan SLC Inc. (Hamamatsu, Japan). The WKY and CP rats were housed in individual cages of uniform size (30 × 40 × 20 cm). The CP-Ex rats were allowed voluntary exercise on a running wheel for 10 weeks. The rats had 24-h free access to the running wheels. The running wheel apparatus included a standard plastic cage (30 × 40 × 20 cm) and a running wheel (diameter, 31.8 cm; width, 10.0 cm) that

was attached to the cage [21]. The number of revolutions per 24-h period was recorded using a computer. All groups were provided the standard diet (3.60 kcal/g, 23.6% protein, 5.3% fat, and 54.4% carbohydrates; Oriental Yeast Co. Ltd., Tokyo, Japan) and water *ad libitum*. The caloric intake of each rat was measured daily. The room was maintained at 22 ± 2°C in a controlled 12-h light/dark cycle (light period from 0800 to 2000) with 45–55% relative humidity. The body weights and systolic and diastolic blood pressure levels of the rats were measured at 5, 7, 9, 11, 13, and 15 weeks of age. Systolic and diastolic blood pressure levels were determined automatically in conscious rats by using the indirect tail-cuff method with a sphygmomanometer (BP-98A, Softron Inc., Tokyo, Japan).

Blood glucose analyses

Glucose levels were measured at 5, 7, 9, 11, 13, and 15 weeks of age after a 15-h fasting period. Blood samples were obtained from the tails of fully conscious rats and analyzed with a glucose meter (GT-1650; Arkray Inc., Kyoto, Japan). The same glucose meter was used to measure non-fasting glucose levels at 15 weeks of age.

Serum biochemical analyses

The CP-Ex rats (15 weeks of age) were placed in cages without running wheels 1 day before their blood was sampled. After fasting glucose levels were measured, the rats were administered an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight), and blood samples were obtained from the abdominal aorta. The serum levels of triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and free fatty acids were measured using routine laboratory methods. The serum levels of insulin, leptin, and high molecular-weight adiponectin were measured using enzyme-

linked immunosorbent assay kits for rats (Shibayagi Co. Ltd., Shibukawa, Japan).

Muscle biochemical analyses

After blood sampling, the soleus muscle was removed from each leg and measured (wet muscle weight). For biochemical and histochemical analyses, the right soleus muscle was divided into distal and proximal components. The distal component was thawed, minced, and homogenized in 5 volumes of ice-cold 0.3 M phosphate buffer (pH 7.4) with a glass homogenizer for the analysis of succinate dehydrogenase (SDH) activity, an indicator of mitochondrial oxidative capacity [22]. The homogenate was diluted further in the same buffer, and 0.04 mL of the homogenate was added to 0.1 mL of sodium succinate solution in a cuvette. After 2 min, 0.1 mL of sodium cyanide (NaCN) was added to the cuvette. The reaction was initiated by adding 2.8 mL of cytochrome *c*-salt solution. The cuvette was transferred to the spectrophotometer; the reduction in cytochrome *c* was evident because of increase in extinction at 550 nm. Then, sodium hydrosulfite was added and the extinction was determined. This reading represented the complete reduction of cytochrome *c*. Sodium succinate was added at a concentration of 17 mM. The final concentrations of the components in the reaction mixture were sodium succinate, 17 mM; NaCN, 1 mM; aluminum chloride ($AlCl_3$), 0.4 mM; calcium chloride ($CaCl_2$), 0.4 mM; cytochrome *c*, 0.017 mM; and phosphate buffer, 0.04 M. The reduction of cytochrome *c* in this reaction mixture was analyzed spectrophotometrically by observing the increase in extinction at 550 nm. The SDH activity was calculated on the basis of ferricytochrome *c* concentration. Protein concentration was determined by the method of Lowry *et al.* [23].

Muscle histochemical analyses

The proximal component of the right muscle was pinned to a corkboard and rapidly frozen in isopentane that had been cooled with a mixture of dry ice and acetone. The muscle was mounted on a specimen chuck with Tissue-Tek OCT compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan). Serial transverse sections were cut at a thickness of 10 μm by using a cryostat at -20°C. The fiber types were classified on the basis of ATPase activity [24, 25]. The sections were brought to room temperature, air dried for 30 min, and preincubated in acidic (pH 4.5) or alkaline (pH 10.4) solution for the subsequent assessment of ATPase activity. The muscle fibers in each section were classified as type I (positive results for ATPase with preincubation at pH 4.5 and negative results for ATPase with preincubation at pH 10.4), type IIA (negative results for ATPase with preincubation at pH 4.5 and positive results for ATPase with preincubation at pH 10.4), and type IIC (positive results for ATPase with preincubation at pH 4.5 and 10.4). A computer-assisted image processing system (Neuroimaging System Inc., Kyoto, Japan) was used to digitize a single common segment selected from each section as gray level images. The cross-sectional area was measured by tracing the outline of each fiber in the section. The fiber-type distribution and cross-sectional area were determined for approximately 500 fibers in the central region of the muscle section.

The sections were stained for 10 min to evaluate SDH staining intensity [24, 25]. The SDH staining intensity was quantified in the 500 fibers by using the computer-assisted image processing system. The sectional images were digitized as gray scale images. Each pixel was quantified as one of 256 gray levels; gray level 0 was equivalent to 100% light transmission, and gray level 255 was equivalent to 0% light transmission. The mean optical density (OD) of all pixels (which were converted to gray level values) within a fiber was determined using a calibration photographic tablet containing 21 gradient-density range steps and corresponding diffused density values.

Muscle mRNA analyses

Total RNA was extracted from the left muscle by using TRIzol Reagent (Invitrogen, Carlsbad, CA) and then treated with deoxyribonuclease I (Invitrogen). The PrimeScript RT reagent kit (Takara Bio Inc., Otsu, Japan) was used to synthesize the first strand of cDNA from 1.0 μg of total RNA. We analyzed gene expression by using real-time polymerase chain reaction with a LightCycler system DX400 (Roche Diagnostics, Mannheim, Germany) and SYBR Premix Ex Taq II (Takara Bio Inc.). The mRNA levels of PPARα, PPARδ/α, PGC-1α, glucose transporter 4 (GLUT4), stearoyl-CoA desaturase-1 (SCD-1), carnitine palmitoyltransferase-I (CPT-I), medium-chain acyl-CoA dehydrogenase (MCAD), and mitochondrial transcriptional factor A (TFAM) were normalized to the mRNA levels of hypoxanthine phosphoribosyltransferase (HPRT). The following primer sets were used:

PPARα forward, 5′-CACCCTCTCTCCAGCTTCCA-3′

PPARα reverse, 5′-GCCTTGTCCCCACATATTCG-3′

PPARδ/β forward, 5′-AACGAGATCAGCGTGCATGTG-3′

PPARδ/β reverse, 5′-TGAGGAAGAGGCTGCTGAAGTT-3′

PGC-1α forward, 5′-CGATGACCCTCCTCACACCA-3′

PGC-1α reverse, 5′-TTGGCTTGAGCATGTTGCG-3′

GLUT4 forward, 5′-CAACTGGACCTGTAACTTCATCGT-3′

GLUT4 reverse, 5′-ACGGCAAATAGAAGGAAGACGTA-3′

SCD-1 forward, 5′-TGGGAAAGTGAAGCGAGCAACCG-3′

SCD-1 reverse, 5′-AGAGGGGCACCTTCTTCATCTTCT C-3′

CPT-I forward, 5′-TCCTACCAGATGGAGAGGATGT-3′

CPT-I reverse, 5′-TAGAGCCAAACCTTGAAGAAGC-3′

MCAD forward, 5′-TGCTGGAAATGATCAACAGAAG-3′

MCAD reverse, 5′-CATCACCCTTCTTCTCTGCTTT-3′

TFAM forward, 5′-GAAACGCCTAAAGAAGAAAGCA-3′

TFAM reverse, 5′-CTGACTCATCCTTAGCCTCC TG-3′

HPRT forward, 5′-CTCATGGACTGATTATGGACAGGAC-3′

HPRT reverse, 5′-GCAGGTCAGCAAAGAACTTATAGCC-3′

Statistical analyses

Standard procedures were used to calculate the mean, standard deviation, and correlation coefficient from individual values. All measured values were presented as mean and standard deviation. One-way analysis of variance was used to determine significant mean differences among the 3 groups. Mean values determined to be significantly different were subjected to additional comparison analysis by using Scheffé's *post hoc* tests. A probability level of 0.05 was considered significant.

Results

Voluntary running distance

Rats in the CP-Ex group ran the greatest distance at 7 weeks of age (Fig. 1). During the 10-week exercise period, the average running distance was 2.3 km/day.

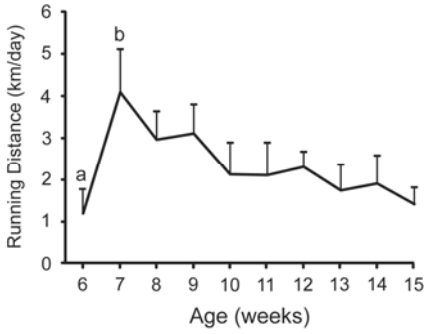

Fig. 1. Voluntary running distances in the CP-Ex group. Data are presented as mean and standard deviation ($n = 6$). [a]$P < 0.05$, compared to 8 and 9 weeks of age; [b]$P < 0.05$, compared to 6 and 10–15 weeks of age.

Body weight and caloric intake

Body weight at 9–15 weeks of age was higher in the CP and CP-Ex groups than in the age-matched WKY group (Fig. 2a). The caloric intake at 5–15 weeks of age was higher in the CP and CP-Ex groups than in the age-matched WKY group (Fig. 2b). Furthermore, the caloric intake at 9–15 weeks of age was higher in the CP-Ex group than in the age-matched CP group.

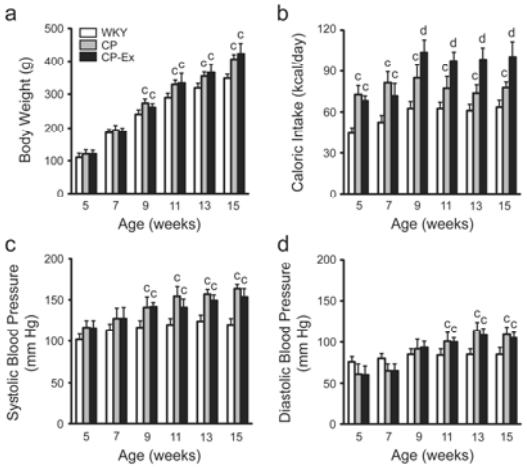

Fig. 2. Body weights (a), caloric intakes (b), and systolic (c) and diastolic (d) blood pressure levels in the WKY, CP, and CP-Ex groups. Data are presented as mean and standard deviation ($n = 6$). $^{c}P < 0.05$, compared to age-matched WKY group; $^{d}P < 0.05$, compared to age-matched WKY and CP groups.

Blood pressure levels

The systolic blood pressure levels at 9–15 weeks of age were higher in the CP and CP-Ex groups than in the age-matched WKY group (Fig. 2c). The diastolic blood pressure levels at 11–15 weeks of age were higher in the CP and CP-Ex groups than in the age-matched WKY group (Fig. 2d).

Blood glucose levels

Fasting glucose levels at 5–7 weeks of age were higher in the CP and CP-Ex groups than in the age-matched WKY group (Fig. 3a). Non-fasting glucose levels at 15 weeks of age were higher in the CP and CP-Ex groups than in the age-matched WKY group (Fig. 3b).

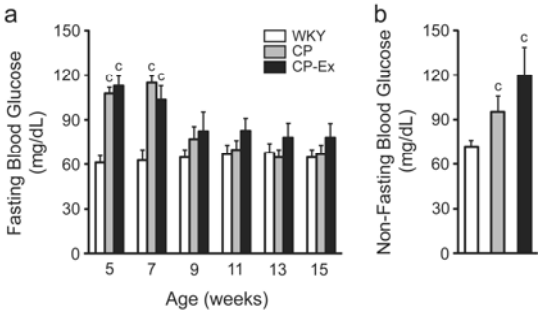

Fig. 3. Fasting (a) and non-fasting (b) blood glucose levels in the WKY, CP, and CP-Ex groups. Data are presented as mean and standard deviation ($n = 6$). $^{c}P < 0.05$, compared to age-matched WKY group.

Serum biochemical parameters

The levels of triglyceride (Fig. 4a), insulin (Fig. 4f), and leptin (Fig. 4g) were higher in the CP and CP-Ex groups than in the WKY group. Total cholesterol levels were higher in the CP group than in the WKY group (Fig. 4b). HDL-cholesterol (Fig. 4c) and adiponectin (Fig. 4h) difference in the levels of LDL-cholesterol (Fig. 4d) or free fatty acids (Fig. 4e) among the WKY, CP, and CP-Ex groups. Triglyceride levels were lower (Fig. 4a) and adiponectin levels higher (Fig. 4h) in the CP-Ex group than in the CP group.

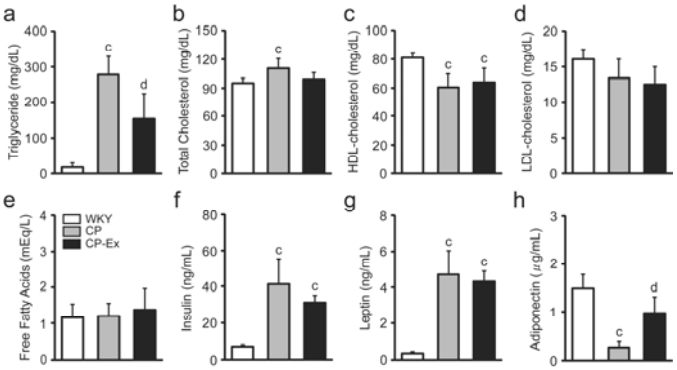

Fig. 4. The levels of triglyceride (a), total cholesterol (b), HDL-cholesterol (c), LDL-cholesterol (d), free fatty acids (e), insulin (f), leptin (g), and adiponectin (h) in the WKY, CP, and CP-Ex groups. Data are presented as mean and standard deviation ($n = 6$). HDL, high-density lipoprotein; LDL, low-density lipoprotein. $^{c}P < 0.05$, compared to WKY group; $^{d}P < 0.05$, compared to WKY and CP groups.

Muscle weight and SDH activity

The muscle weight (Fig. 5a) and relative muscle weight per body weight (Fig. 5b) were lower in the CP group than in the WKY and CP-Ex groups. Muscle SDH activity was lower in the CP group than in the WKY and CP-Ex groups (Fig. 5c).

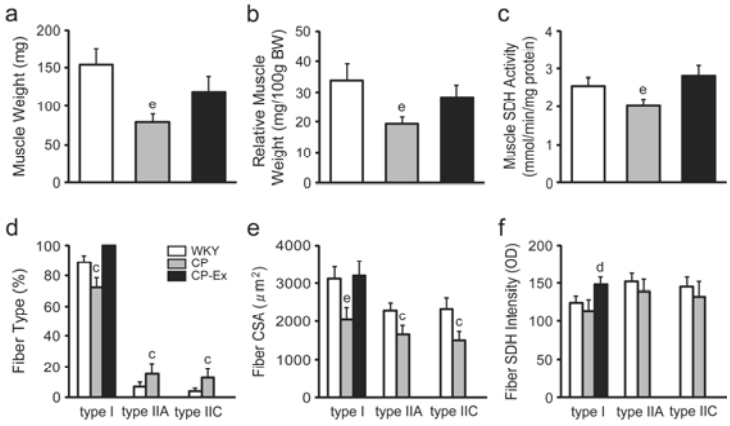

Fig. 5. Soleus muscle weights (a), relative muscle weights per body weight (b), muscle SDH activities (c), fiber type percentages (d), fiber cross-sectional areas (e), and fiber SDH staining intensities (f) in the WKY, CP, and CP-Ex groups. Data are presented as mean and standard deviation (n = 6). BW, body weight; SDH, succinate dehydrogenase; CSA, cross-sectional area; OD, optical density. $^{c}P < 0.05$, compared to WKY group; $^{d}P < 0.05$, compared to WKY and CP groups; $^{e}P < 0.05$, compared to WKY and CP-Ex groups.

Muscle fiber profiles

The muscles in WKY and CP rats contained 3 types of fibers (Fig. 6). In contrast, the muscles in CP-Ex rats contained only type I fibers. The percentage of type I fibers was lower and the percentages of type IIA and IIC fibers were higher in the CP group than in the WKY group (Fig. 5d). The cross-sectional area of type I fibers was smaller in the CP group than in the WKY and CP-Ex groups (Fig. 5e). The cross-sectional areas of type IIA and IIC fibers were smaller in the CP group than in the WKY group. The SDH staining intensity of type I fibers was higher in the CP-Ex group than in the WKY and CP groups (Fig. 5f).

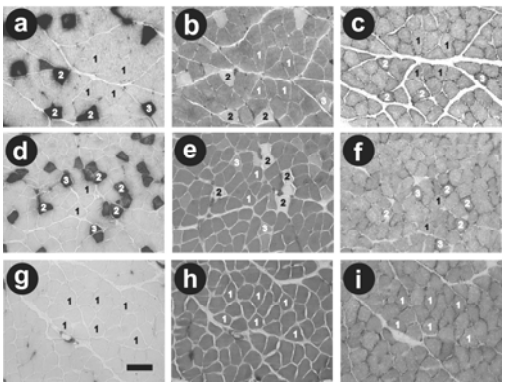

Fig. 6. Serial transverse sections of the soleus muscles from WKY (a–c), CP (d–f), and CP-Ex (g–i) rats stained for ATPase activity after preincubation at pH 10.4 (a, d, and g) and pH 4.5 (b, e, and h) and for SDH activity (c, f, and i). 1, type I; 2, type IIA; and 3, type IIC. Scale bar on g = 100 μm.

Muscle mRNA levels

PPARα mRNA levels were higher (Fig. 7a) and PPARδ/β and PGC-1α mRNA levels lower (Fig. 7b, c) in the CP group than in the WKY and CP-Ex groups. SCD-1 mRNA levels were higher in the CP and CP-Ex groups than in the WKY group (Fig. 7e). There was no difference in the mRNA levels of GLUT4 (Fig. 7d), CPT-I (Fig. 7f), MCAD (Fig. 7g), or TFAM (Fig. 7h) among the WKY, CP, and CP-Ex groups.

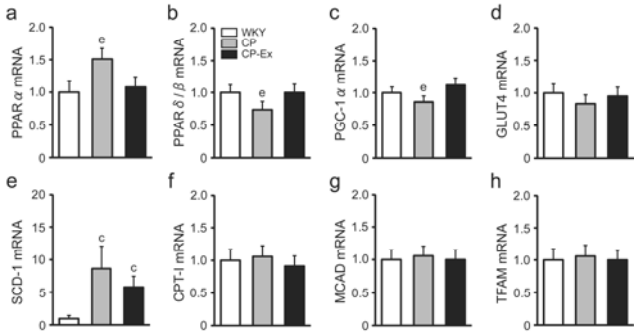

Fig. 7. The mRNA levels of PPARα (a), PPARδ/β (b), PGC-1α (c), GLUT4 (d), SCD-1 (e), CPT-I (f), MCAD (g), and TFAM (h) in the soleus muscles of the WKY, CP, and CP-Ex groups. Data are presented as mean and standard deviation (n = 6). PPAR, peroxisome proliferator-activated receptor; PGC-1α, PPARγ coactivator-1α; GLUT4, glucose transporter 4; SCD-1,stearoyl-CoA desaturase-1; CPT-I, carnitine palmitoyltransferase-I; MCAD, medium-chain acyl-CoA dehydrogenase; TFAM, mitochondrial transcriptional factor A. $^{c}P < 0.05$, compared to WKY group; $^{e}P < 0.05$, compared to WKY and CP-Ex groups.

Relationships between running distance and muscle SDH activity and PGC-1α mRNA levels

The running distance of individual rats in the CP-Ex group correlated positively with muscle SDH activity (Fig. 8a) and PGC-1α mRNA levels (Fig. 8b).

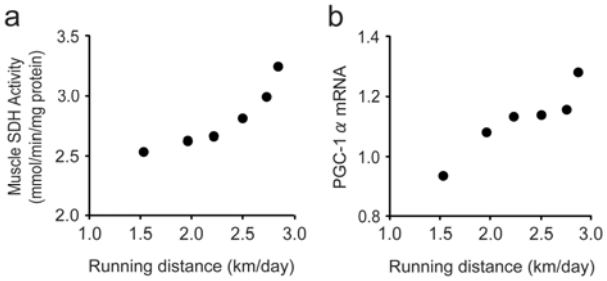

Fig. 8. Relationships between running distance and SDH activity (r = 0.90, n = 6, $P < 0.01$; a) and PGC-1α mRNA levels (r = 0.90, n = 6, $P < 0.01$; b) in the soleus muscles of the CP-Ex group. SDH, succinate dehydrogenase.

Discussion

Obesity, defined as an increase in adipose tissue mass, is associated with various metabolic diseases. CP rats, which are used as an animal model for metabolic syndrome, were established from an inbred subline of obese, spontaneously hypertensive SHR/N-cp (*cp*/*cp*) rats [19, 20]. CP rats are characterized by risk factors for metabolic syndrome, including high blood pressure and glucose levels, hyperinsulinemia, and dyslipidemia. The 15-week-old CP rats used in the present study showed signs of metabolic syndrome, including increased body weight (Fig. 2a); high systolic and diastolic blood pressure levels (Fig. 2c, d); and increased levels of glucose (Fig. 3b), triglyceride (Fig. 4a), and insulin (Fig. 4f).

Limited data are available on the skeletal muscle characteristics of patients and animals with metabolic syndrome under various conditions, such as excessive caloric intake and/or decreased muscle activity. In the present study, we compared the fiber characteristics and mRNA levels in the soleus muscles of CP rats that were permitted running exercise to those of non-obese WKY and obese CP rats.

At 9–15 weeks of age, the CP-Ex group showed greater caloric intake than the CP group (Fig. 2b). However, the 2 groups showed similar body weight at all ages (Fig. 2a). CP-Ex rats may have maintained sufficient energy expenditure and oxidative metabolism to complete their daily exercise. Rats are highly active and exercise spontaneously on running wheels at their own pace that allows them to run greater distances than what is normally observed during forced treadmill exercise regimens. Voluntary running primarily induces aerobic adaptation in the skeletal muscles [26–28], and fiber hypertrophy can develop in the soleus muscles of rats [26, 29]. In the present study, the average running distance per day in the CP-Ex group was relatively low (2.3 km/day), compared to that (about 16 km/day) noted in previous studies on normal rats [26, 29], and that (about 5 km/day) in our previous study on

normal rats with the same running wheel apparatus [30]. However, in the present study, the skeletal muscles (Fig. 5c) and fibers (Fig. 5f) showed higher oxidative enzyme activity in response to exercise. This indicated that the CP-Ex group expended sufficient energy to improve the oxidative metabolism in skeletal muscles.

Insulin and leptin stimulate the sympathetic system [31, 32], and this contributes to increased blood pressure. In the present study, the CP and CP-Ex groups exhibited high systolic (Fig. 2c) and diastolic (Fig. 2d) blood pressure levels and increased levels of insulin (Fig. 4f) and leptin (Fig. 4g), compared to those exhibited by the WKY group. In contrast, running exercise restored adiponectin levels, which had decreased in the rats with metabolic syndrome (Fig. 4h). Low adiponectin levels are associated with subsequent development of cardiovascular disease and type 2 diabetes [33]. In the present study, we showed that a 10-week of running exercise did not induce any changes in the levels of glucose (Fig. 3a) or insulin (Fig. 4f) of the CP-Ex group. However, running exercise did increase adiponectin levels (Fig. 4h). Therefore, we speculate that insulin sensitivity would improve further with continued running exercise.

The CP group exhibited lower soleus muscle weight than the WKY group (Fig. 5a, b). The soleus muscle is an antigravity and postural muscle that is continually active and therefore, more susceptible to decreased physical activity. In contrast, the soleus muscle weight (Fig. 5a, b) and cross-sectional area of type I fiber (Fig. 5e) in the CP-Ex group were greater than those in the CP group and similar to those in the WKY group. Therefore, we concluded that lower muscle weight in the CP group was associated with lower physical activity and loading levels.

The soleus muscles in CP rats contained type I, IIA, and IIC fibers, whereas those in CP-Ex rats contained only high-oxidative type I fibers (Figs. 5d, 6). These findings suggest that

running exercise induce a shift of fibers from types IIA and IIC to high-oxidative type I. In the present study, a complete shift of all fibers to type I in the soleus muscles of the CP-Ex group was observed. Fiber type shifts occur under a variety of conditions, such as growth, aging, disease, and increased or decreased muscle activity [34]. To our knowledge, no previous studies have reported the shift of all fibers to a single type—with the exception of our finding that the soleus muscles in adult rats with type 2 diabetes contain only type I fibers that have lower oxidative enzyme activity than type IIA and IIC fibers [9–12]. However, the mechanism underlying the complete shift of fibers to a single type in the soleus muscles of CP-Ex rats remains unclear.

Previous studies [35, 36] revealed that hypertriglyceridemia and insulin resistance are associated with increased SCD-1 activity. The present study showed that SCD-1 mRNA levels were 8.6- and 5.8-fold greater in the CP and CP-Ex groups, respectively, than in the WKY group (Fig. 7e). We concluded that hypertriglyceridemia (Fig. 4a) and hyperinsulinemia (Fig. 4f) observed in the CP and CP-Ex groups were associated with increased SCD-1 mRNA levels.

PGC-1α mRNA levels in the soleus muscles were higher in the CP-Ex group than in the CP group (Fig. 7c). In addition, there was no difference in PGC-1α mRNA levels between the WKY and CP-Ex groups (Fig. 7c). It is suggested that running exercise restored decreased PGC-1α mRNA levels and improved the oxidative capacity of skeletal muscles in rats with metabolic syndrome. In fact, the oxidative capacity of the soleus muscles (Fig. 5c) and fibers (Fig. 5f) was higher in the CP-Ex group than in the CP group.

Voluntary running distance correlated positively with muscle SDH activity (Fig. 8a) and PGC-1α mRNA levels (Fig. 8b). The rats with metabolic syndrome that ran longer distances exhibited increased muscle oxidative capacity, indicating that adequate physical activity

enhances muscle oxidative capacity in rats with metabolic syndrome. The oxidative capacity in skeletal muscles may be a key factor that influences the progression or prevention of metabolic syndrome.

Conclusion

The development and progression of metabolic syndrome depend on genetic background but are also highly influenced by daily lifestyle. CP rats possess genetic factors that easily induce metabolic syndrome, however, running exercise prevents decrease in muscle oxidative capacity and PGC-1α mRNA levels. Furthermore, longer running distances correlate with higher muscle oxidative enzyme activity and PGC-1α mRNA levels. We concluded that running exercise restores decreased muscle oxidative capacity and PGC-1α mRNA levels and improves hypertriglyceridemia in rats with metabolic syndrome.

Acknowledgments Fumiko Nagatomo is a Research Fellow of the Japan Society for the Promotion of Science. This study was supported by the Japan Aerospace Exploration Agency. The authors declare that they have no conflict of interest.

References

1. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS (2003) Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA 289: 76–79
2. Reaven GM (2006) The metabolic syndrome: is this diagnosis necessary? Am J Clin Nutr 83: 1237–1247
3. Ballantyne CM, Hoogeveen RC, McNeill AM, Heiss G, Schmidt MI, Duncan BB, Pankow

JS (2008) Metabolic syndrome risk for cardiovascular disease and diabetes in the ARIC study. Int J Obes 32: S21–S24

4. Kelly DE, Goodpaster BH, Wing RR, Simoneau JA (1999) Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol Endocrinol Metab 277: E1130–E1141
5. Mårin P, Andersson B, Krotkiewski M, Björntorp P (1994) Muscle fiber composition and capillary density in women and men with NIDDM. Diabetes Care 17: 382–386
6. Hickey MS, Carey JO, Azevedo JL, Houmard JA, Pories WJ, Israel RG, Dohm GL (1995) Skeletal muscle fiber composition is related to adiposity and in vitro glucose transport rate in humans. Am J Physiol Endocrinol Metab 268: E453–E457
7. Nyholm B, Qu Z, Kaal A, Pedersen SB, Gravholt CH, Andersen JL, Saltin B, Schmitz O (1997) Evidence of an increased number of type IIb muscle fibers in insulin-resistant first-degree relatives of patients with NIDDM. Diabetes 46: 1822–1828
8. Gaster M, Staehr P, Beck-Nielsen H, Schrøder HD, Handberg A (2001) GLUT4 is reduced in slow muscle fibers of type 2 diabetic patients: is insulin resistance in type 2 diabetes a slow, type 1 fiber disease? Diabetes 50: 1324–1329
9. Yasuda K, Ishihara A, Adachi T, Shihara N, Seino Y, Tsuda K (2001) Growth-related changes in skeletal muscle fiber type and insulin resistance in Diabetic Otsuka Long-Evans Tokushima fatty rats. Acta Histochem Cytochem 34: 371–382
10. Yasuda K, Nishikawa W, Iwanaka N, Nakamura E, Seino Y, Tsuda K, Ishihara A (2002) Abnormality in fibre type distribution of soleus and plantaris muscles in non-obese diabetic Goto-Kakizaki rats. Clin Exp Pharmacol Physiol 29: 1001–1008
11. Yasuda K, Adachi T, Gu N, Matsumoto A, Matsunaga T, Tsujimoto G, Tsuda K, Ishihara A (2007) Effects of hyperbaric exposure with high oxygen concentration on glucose and

insulin levels and skeletal muscle-fiber properties in diabetic rats. Muscle Nerve 35: 337–343

12. Gu N, Nagatomo F, Fujino H, Takeda I, Tsuda K, Ishihara A (2010) Hyperbaric oxygen exposure improves blood glucose level and muscle oxidative capacity in rats with type 2 diabetes. Diabetes Technol Ther 12: 125–133
13. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM (2002) Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. Nature 418: 797–801
14. Wende AR, Huss JM, Schaeffer PJ, Giguère V, Kelly DP (2005) PGC-1α coactivates PDK4 gene expression via the orphan nuclear receptor ERRα: a mechanism for transcriptional control of muscle glucose metabolism. Mol Cell Biol 25: 10684–10694
15. Schuler M, Ali F, Chambon C, Duteil D, Bornert JM, Tardivel A, Desvergne B, Wahli W, Chambon P, Metzger D (2006) PGC-1α expression is controlled in skeletal muscles by PPARβ, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. Cell Metab 4: 407–414
16. Nagatomo F, Gu N, Fujino H, Takeda I, Tsuda K, Ishihara A (2009) Skeletal muscle characteristics of rats with obesity, diabetes, hypertension, and hyperlipidemia. J Atheroscler Thromb 16: 576–585
17. Nagatomo F, Fujino H, Kondo H, Gu N, Takeda I, Ishioka N, Tsuda K, Ishihara A (2011) PGC-1α mRNA level and oxidative capacity of the plantaris muscle in rats with metabolic syndrome, hypertension, and type 2 diabetes. Acta Histochem Cytochem 44: 73–80
18. Hennig R, Lømo T (1985) Firing patterns of motor units in normal rats. Nature 314: 164–

166

19. Takaya K, Ogawa Y, Hiraoka J, Hosoda J, Yamori Y, Nakao K (1996) Nonsense mutation of leptin receptor in the obese spontaneously hypertensive Koletsky rat. Nat Genet 14: 130–131

20. Friedman JE, Ishizuka T, Liu S, Farrell CJ, Bedol D, Koletsky RJ, Kaung HL, Ernsberger P (1997) Reduced insulin receptor signaling in the obese spontaneously hypertensive Koletsky rat. Am J Physiol Endcrinol Metab 273: E1014–E1023

21. Ishihara A, Roy RR, Ohira Y, Ibata Y, Edgerton VR (1998) Hypertrophy of rat plantaris muscle fibers after voluntary running with increasing loads. J Appl Physiol 84: 2183–2189

22. Cooperstein SJ, Lazarow A, Kurfess NJ (1950) A microspectrophotometric method for the determination of succinate dehydrogenase. J Biol Chem 186: 129–139

23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

24. Nakatani T, Nakashima T, Kita T, Ishihara A (2003) Cell size and oxidative enzyme activity of type-identified fibers in rat hindlimb muscles: a review. Acta Histochem Cytochem 36: 105–114

25. Nagatomo F, Fujino H, Kondo H, Suzuki H, Kouzaki M, Takeda I, Ishihara A (2011) PGC-1α and FOXO1 mRNA levels and fiber characteristics of the soleus and plantaris muscles in rats after hindlimb unloading. Histol Histopathol 26: 1545–1553

26. Rodnick KJ, Reaven GM, Haskell WL, Sims CR, Mondon CE (1989) Variations in running activity and enzymatic adaptations in voluntary running rats. J Appl Physiol 66: 1250–1257

27. Lambert MI, Noakes TD (1990) Spontaneous running increases Vo_{2max} and running

performance in rats. J Appl Physiol 68: 400–403

28. Sexton WL (1995) Vascular adaptations in rat hindlimb skeletal muscle after voluntary running-wheel exercise. J Appl Physiol 79: 287–296

29. Munoz KA, Aannestad A, Tischler ME, Henriksen EJ (1994) Skeletal muscle protein content and synthesis after voluntary running and subsequent unweighting. Metabolism 43: 994–999

30. Matsumoto A, Okiura T, Morimatsu F, Ohira Y, Ishihara A (2007) Effects of hyperbaric exposure with high oxygen concentration on the physical activity of developing rats. Dev Neurosci 29: 452–459

31. Reaven GM, Lithell H, Landsberg L (1996) Hypertension and associated metabolic abnormalities—the role of insulin resistance and the sympathoadrenal system. N Engl J Med 334: 374–381

32. Landsberg L (2001) Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). J Hypertens 19: 523–528

33. Lim S, Koo BK, Cho SW, Kihara S, Funahashi T, Cho YM, Kim SY, Lee HK, Shimomura I, Park KS (2008) Association of adiponectin and resistin with cardiovascular events in Korean patients with type 2 diabetes: the Korean atherosclerosis study (KAS): a 42-month prospective study. Atherosclerosis 196: 398–404

34. Edgerton VR, Bodine-Fowler S, Roy RR, Ishihara A, Hodgson JA (1996) Neuromuscular adaptation. In: Rowell LB and Shepherd JT (ed) Handbook of Physiology. Section 12: Exercise: Regulation and Integration of Multiple Systems. Oxford University Press New York, 55–88

35. Gbaguidi FG, Chinetti G, Milosavljevic D, Teissier E, Chapman J, Olivecrona G, Fruchart

JC, Griglio S, Fruchart-Najib J, Staels B (2002) Peroxisome proliferator-activated receptor (PPAR) agonists decrease lipoprotein lipase secretion and glycated LDL uptake by human macrophages. FEBS Lett 512: 85–90

36. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F (1995) Effects of the obese gene product on body weight regulation on ob/ob mice. Science 269: 540–543